Attorney Docket: VJP–1030–US
(Formerly 10013.0014US)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:
Thomas Schmidt

Confirmation Number: 1170

Application No.: 10/026,578

Group Art Unit: 1652

Filed: December 17, 2001

Examiner: Hope A. Robinson

Title: SEQUENTIALLY ARRANGED STREPTAVIDIN–BINDING MODULES AS AFFINITY TAGS

DECLARATION OF DR. THOMAS SCHMIDT

I, Thomas Schmidt state and declare as follows:

1. I am currently Chief Operating Officer of IBA GmbH, the assignee of the above-referenced '578 application. I am named as the sole inventor of the '578 Patent Application. I have been practicing molecular biology techniques, including the expression of fusion proteins using affinity tagging, for over 18 years. A copy of my *curriculum vitae* is attached to this declaration.

2. I have read and am familiar with the most recent Office Action issued in the '578 Patent Application, which was mailed November 24, 2008. Hereinafter I will refer to this as the "Office Action."

3. I note that the Written Description and Enablement rejections, and the remarks directed thereto, do not comport with the knowledge available to the skilled artisan concerning the use of N- and C-terminal affinity tags as part of a fusion protein.

4. The Office Action is unduly focused on the overall structure of the fusion protein in its criticism of the claimed invention. For example, on page 3, the Office Action offers that

"the claims are directed to a streptavidin binding peptide linked to any protein" and so "a skilled artisan cannot envision the detailed chemical structure of the fusion protein as claimed". Similarly, the Office Action remarks on pages 5 and 6 that "no structure is provided for said fusion protein to make the correlation between structure and function," and "[a] skilled artisan cannot predict that any known or unknown protein would bind to streptavidin." Such remarks appear to be made from the point of view of one who has no experience in the use of such affinity tags.

5. I refer to Ford *et al., Prot. Expr. Purific.*, 2, 95–107, 1991 for a review of the field regarding fusion tags as it stood more than a decade before the filing date of the present invention. As noted therein, numerous "fusion tail systems have been developed to promote efficient recovery and purification of recombinant proteins from crude cell extracts or culture media. In these systems, a target protein is genetically engineered to contain a C- or N-terminal polypeptide tail, which provides the biochemical basis for specificity in recovery and purification." Ford *et al.*, Abstract. Such affinity tags "have been designed for fusion to virtually any target protein that can be cloned and expressed in a microbial host." Ford *et al.*, page 95, right column. As noted in Fig. 1 of Ford *et al.*, affinity for the binding partner of the affinity tag is provided by the affinity tag itself; the remainder of the fusion protein is largely irrelevant to this interaction.

6. Thus, the focus of the Office Action on the unknown nature of the remainder of the fusion protein (discussed above in Paragraph 4 of this Declaration) is essentially irrelevant to one of skill in the art. The relevant question is whether the affinity tag has the requisite binding affinity.

7. I note that the Examiner has cited Skerra *et al.*, U.S. Patent No. 5,506,121, which discloses the use of a single peptide having the sequence Trp–X–His–Pro–Gln–Phe–Y-Z as an affinity tag, as allegedly anticipating the invention claimed in the present invention. The present patent application describes and claims the use of a sequential arrangement of two modules with an amino acid sequence of –His–Pro–Baa– in which Baa is selected from the group consisting of glutamine, asparagine and methionine. At least one of the modules comprises a sequence –His–Pro–Gln–Phe–. The Examiner states that Skerra *et al.* "outlines that 'peptides' are made and

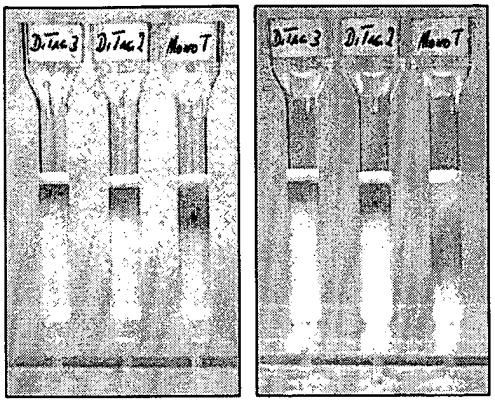
fused and they are normally 3 to 12 amino acids in length." Office Action, page 11. I note, however, that the section of Skerra *et al.* to which the Examiner refers has nothing to do with the use of sequential fusion tags (affinity tags). Rather, it is a factual statement in the background section of Skerra *et al.* about certain previously known fusion/affinity tags:

> Examples of such peptide tags are the Myc-tag (Munro & Pelham (1986) Cell 46, 291-300; Ward et al. (1989) Nature 341, 544-546), the Flag peptide (Hopp et al. (1988) Bio/Technology 6, 1204-1210), the KT3 epitope peptide (Martin et al. (1990) Cell 63,843-849; Martin et al. (1992) Science 255, 192-194), an α-tubulin epitope peptide (Skinner et al. (1991) J. Biol. Chem. 266, 14163-14166) and the T7 gene 10-protein peptide tag (Lutz-Freyermuth et al. (1990) Proc. Natl. Acad. Sci. USA 87, 6393-6397) which have been used successfully for the detection and in some cases also for the purification of the recombinant gene product. In addition it was found that in most of the aforementioned examples these short peptide tags, which are normally 3 to 12 amino acids long, do not interfere with the biological function of the protein and therefore do not necessarily have to be cleaved after expression.

Skerra *et al.*, column 2, lines 36-51(emphasis added). There is no disclosure in Skerra *et al.*, of using any sequential fusion tags, notwithstanding the Examiner's opinion to the contrary. Similarly, while the Examiner has cited Szostak *et al.*, U.S. Patent No. 6,841,359 as allegedly anticipating the invention claimed in the present invention, there is no disclosure in Szostak *et al.*, of using any sequential fusion tags as defined in the claims, in particular no disclosure of a sequential fusion tag comprising a module comprising a sequence –His–Pro–Gln–Phe–. It is my opinion that neither of these cited references discloses the use of the claimed sequential arrangement of fusion tags.

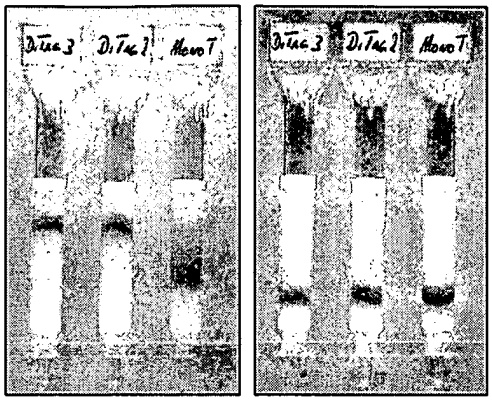
8. The present invention is drawn to improved fusion proteins and peptides able to selectively bind to substrates comprising streptavidin (which term means either or both naturally-occurring and mutein or optimized forms such as the commercially available STREP-TACTINS®). The streptavidin-binding peptides of the present invention have the ability to bind strongly to their substrate under non-competitive conditions and yet could be easily displaced under competitive conditions. This bimodal characteristic results from cooperative binding of a sequential arrangement in the peptide of at least two binding modules, wherein each module is able to independently bind either streptavidin or a streptavidin mutein. In this way, the multiple modules cooperate under non-competitive conditions in a synergistic manner to cause tight

binding of the peptide to the substrate. This is depicted in the following figures, which are presented in the present specification as Fig. 1:



In this figure, the left panel depicts binding to a streptavidin matrix of a fusion protein (a red coloured cytochrome in this case) having a sequential arrangement of two peptide tags (left and center column where the two peptide tags are separated by different linker sequences; *i.e.* GGGSGGGSGGGS for Di-tag3 and GGGSGGGS for Di-tag2 respectively) of the present invention, compared to a single peptide tag (right column). The right panel depicts washing of the column with a buffer. It is noted that the fusion peptide having a single tag begins to wash through the column, while the sequential tags bind more effectively.

9. Under competitive elution conditions, however, each module of the sequential tags has to compete independently with binding of a free substrate binding molecule (or mimic thereof), *e.g.* biotin or biotin derivatives, thereby resulting in the effect that elution of the whole peptide that comprises the two sequentially binding modules is almost as fast as for a single module alone:



In this figure, the left panel depicts elution from the streptavidin matrix of the fusion protein having a sequential arrangement of two peptide tags (left and center column where the two peptide tags are separated by different linker sequences) of the present invention, compared to a single peptide tag (right column). As seen in the right panel, the fusion peptides having sequential tags elute with similar efficiency as the single tag fusion protein, as all elute at the same solvent front.

10. As described in the present specification (*e.g.*, in pages 3-8 and Example 3), the use of this sequential arrangement provides a surprising and substantial improvement in purification yield relative to the use of a single affinity tag under conservation of mild conditions for efficient elution by competitive displacement. This improvement in yield under preservation of mild conditions for the whole process has unquestionable practical benefit in the purification of recombinantly expressed proteins which often are labile and prone to denaturation under non-physiological conditions.

11. Furthermore, the surprising nature of this discovery is apparent from Szostak *et al.*, U.S. Patent No. 6,841,359, cited by the Examiner in the present case. In column 15, lines 63-66, the '359 patent reports that the presence of two HPQ (His–Pro–Gln) motifs does not confer high affinity binding. Likewise, column 10, lines 12-24 reports that binding to streptavidin is actually conferred by the entirety of a 38 residue peptide. In contrast, the present invention demonstrates that a much simpler sequential arrangement of binding motifs can provide sufficient binding affinity while maintaining the ability of the streptavidin binding peptides to be

readily eluted under competitive mild conditions, and thus can provide improved purification yields.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements so made are punishable by fine or imprisonment or both under § 1001 of Capital Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

May 20, 2009
DATE

DR. THOMAS SCHMIDT

Thomas Schmidt
Dr. phil. nat.

Tabakswinkel 8
D-37139 Adelebsen
Tel: +49 5506 999960
Mobil: +49 163 5067215
Mailto: tgmschmidt@imail.de

Curriculum vitae

Professional career

Since 4/02	Chief Operating Officer of IBA GmbH, Göttingen, Germany, head of research and development and production as well as of corporate patenting and in-licensing.
6/01 -3/02	Acting partner/interim CEO of IBA GmbH, Göttingen.
7/98 - 5/01	Assistant managing director of Dr. H. Stadler, CEO of IBA GmbH until May 2001.
7/94 - 5/01	Development of the business units "Protein TAGnologies" and "Cell TAGnologies" of IBA GmbH. During that time: Development of a comprehensive product portfolio of *Strep*-tag technology for the expression, detection, purification and assay of recombinant proteins and of Streptamer technology for the purification of antigen specific T cells. Acquisition and performance of government-funded research projects. Custom production of recombinant proteins. Lecturer of seminars in the field of recombinant protein expression and purification for postgraduate professional education.

University

1/95	Oral part of the doctoral examination; Completion of PhD thesis Title: "Dr. phil. nat. im Fach Biochemie" (magna cum laude)
2/91 - 6/94	Doctoral research fellowship in biochemistry at the Max-Planck-Institute for Biophysics in Frankfurt/Main, Germany, headed by Prof. Dr. H. Michel (Nobel Prize laureate), in the group of Prof. Dr. A. Skerra in the field of protein engineering/molecular recognition. Development of Strep-tag technology for the detection and purification of recombinant proteins.
2/91	Completion of master of sciences
1/90 - 1/91	Masters thesis at Genzentrum of Ludwig Maximilian University München, Germany in the group of Prof. Dr. A. Plückthun in the field of molecular engineering of recombinant antibody fragments.

Thomas Schmidt
Dr. phil. nat.

Tabakswinkel 8
D-37139 Adelebsen
Tel: +49 5506 999960
Mobil: +49 163 5067215
Mailto: tgmschmidt@imail.de

Publications, posters and patents

Articles

Schmidt, T.G.M. & Skerra, A. (2007) The Strep-tag system for one-step purification and high-affinity detection or capturing of proteins. *Nature Protocols* 2, 1528-1535.

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Dübel, S., Breitling, F., Kontermann, R., **Schmidt, T.G.M.**, Skerra, A. & Little, M. (1995). Bifunctional and multimeric complexes of streptavidin fused to single chain antibodies (scFv). *J. Immunological Methods* 178, 201-209.

Schmidt, T. G. M. & Skerra, A (1994). One-step purification of bacterially produced proteins by means of the "Strep tag" and immobilized recombinant core streptavidin. *J. Chromatogr. A* 676, 337-345.

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Glockshuber, R., **Schmidt, T.G.M.** & Plückthun, A. (1992). The disulfide bonds in antibody variable domains: Effects on stability, folding in vitro, and functional expression in *Escherichia coli*. *Biochemistry* 31, 1270-1279.

Reviews/book contributions

Müller, H. N. & **Schmidt, T.G.M.** (2000). Simple and fast one-step purification of recombinant proteins using the unique *Strep*-tag technology. in: M. Kastner (Ed.), Protein Liquid Chromatography, Elsevier, ISBN: 0-444-50210-6, S. 825-837.

Schmidt, T.G.M. & Skerra, A. (2000). Protein engineering for affinity purification: the *Strep*-tag. in: L. Alberghina (Ed.), Protein Engineering in Industrial Biotechnlogy, Harwood Academic Publishers, ISBN: 90-5702-412-8, S. 41-61.

Skerra, A. & **Schmidt, T.G.M.** (1999). Applications of a peptide ligand for streptavidin: the *Strep*-tag. *Biomolecular Engineering* 16, 79-86.

Schmidt, T.G.M. (1994). Generierung und Charakterisierung künstlicher Peptid-Streptavidin-erkennung: Entwicklung eines C-terminalen Affinitätspeptids für den Nachweis und die Reinigung rekombinanter Proteine. Dissertation an der Johann Wolfgang Goethe-Universität, Frankfurt/Main. Zugleich: Cuvillier, Göttingen 1996, ISBN 3-89588-449-9.

Thomas Schmidt
Dr. phil. nat.

Tabakswinkel 8
D-37139 Adelebsen
Tel: +49 5506 999960
Mobil: +49 163 5067215
Mailto: tgmschmidt@imail.de

Posters

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Patents

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